

## Pollination Systems Inferred from Pollen - Ovule Ratios of Some Species of Podostemaceae

HIROSHI OKADA<sup>1</sup> and MASAHIRO KATO<sup>2</sup>

<sup>1</sup>Botanical Gardens, Faculty of Science, Osaka City University, 2000 Kisaichi, Katano, Osaka 576-0004, Japan; <sup>2</sup>Department of Biological Sciences, Graduate School of Science, University of Tokyo, 7-3-1 Hongo, Tokyo 113-0033, Japan

Members of the Podostemaceae grow submerged in rapidly flowing water in the rainy season, but are exposed and flower and eventually die in the dry season. Pollen - ovule ratios (P/O) of 20 species in 15 genera in the family were calculated and pollination in some species was observed. We observed that autogamous species have low values of log P/O, while allogamous species have high values. We therefore conclude that species with values of log P/O higher than 2.5 are allogamous, while those with a ratio lower than 2.0 are autogamous. The species of the subfamilies Weddellinoideae and Tristichioideae are inferred to be autogamous. The subfamily Podostemoideae consists of species with various pollination types ranging from allogamy to autogamy and cleistogamy. The ancestor of the family that invaded the specialized aquatic habitat may have been autogamous.

Key words: Podostemaceae, pollen - ovule ratio, pollination system

The Podostemaceae are aquatic angiosperms that grow on water-worn rocks in waterfalls and rapids in rivers under seasonally fluctuating water levels. The plants grow submerged in the rainy season, flower and set fruits in the air in the dry season, then become desiccated and die. In the dry season the plants come into simultaneous bloom shortly after they are exposed to the air. The plants of most species consist of adhering, prostrate, cylindrical or foliose roots with vegetative or flowering shoots. The flowers are usually small and inconspicuous, particularly in the Asian species, with reduced tepals and a single ovary. Relatively conspicuous flowers are seen in some American species, e.g., in *Mourera* the flowers are borne in an elongate inflorescence and in *Mourera* and *Rhyncholaxis*

the flowers have numerous stamens and colorful instead of inconspicuous tepals. The floral characters, as in other angiosperm families, are useful for classifying the subfamilies and genera of Podostemaceae (van Royen 1951, 1953, 1954, Cusset & Cusset 1988, Cusset 1992, Cook 1996, Rutishauser 1997). The sepals are relatively large and five in *Weddellina* (subfamily Weddellinoideae) and three in *Dalzellia*, *Indotristicha*, *Malaccotristicha*, *Terniopsis*, and *Tristicha* (Tristichioideae). The tepals are rudimentary in Podostemoideae, but the stamens are variable in number, ranging from one to a few in Asian Podostemoideae and to many in many American species of the subfamily (see also Kita & Kato 2001). The variation in these floral and vegetative characters is reflected in the cur-

rent classification in which about 270 species of the family Podostemaceae are classified into about 47 genera, most of which are monotypic (about 20 genera) or oligotypic (about 20 genera) (Cook 1996).

Pollination systems affect population structure, genetic diversity of populations, and consequently speciation (Stebbins 1974, Crepet & Friis 1987, Endress 1994, Lloyd & Barrett 1996, Proctor *et al.* 1996, Richards 1997, Owens & Rudall 1998, Dafni *et al.* 2000). The unique habitat and specialized morphology of Podostemaceae, as described above, may be strongly correlated with the floral morphology and pollination systems in the family (Sculthorpe 1967). In a comprehensive morphological and ecological study of Indian and Sri Lankan species, Willis (1902) described the flowers as anemophilous (wind pollinated) and probably largely self pollinated in *Indotristicha ramosissima* (Tristichoideae; see also Rutishauser & Huber 1991), *Farmeria metzgerioides*, *Griffithiella* (= *Cladopus*) *hookeriana*, *Polypleurum elongatum*, *P. stylosum*, *Willisia selaginoides*, *Zeylanidium subulatum*, *Z. lichenoides*, and *Z. olivaceum* (Podostemoideae), and fertilized underwater and cleistogamous (even though not strictly so) in *Dalzellia zeylanica* (Tristichoideae) and *Z. barberi*. Möller (1899) suspected cleistogamy in *Cladopus nymanii* and Imamura (1928) described *C. japonicus* as cleistogamous, indicating apogamous reproduction. Philbrick (1984) reported that *Podostemum ceratophyllum* was autogamous and cleistogamous. In an experimental study Philbrick & Novelo (1998) showed that *Marathrum rubrum* was generally autogamous but rarely outcrossing.

This study describes results of field observations of pollination in a few species and the pollen/ovule ratio (P/O) of 20 species assigned to 15 genera in three subfamilies. The P/O ratio reflects pollination efficiency or the likelihood of

pollen grains reaching a stigma (Cruden 1977, 2000). Cruden (1977, 2000) has shown that outcrossing flowers have higher P/O ratios than selfing flowers, and selfing flowers have higher P/O ratios than cleistogamous flowers, although there is considerable variation and not all species fit such a rule (e.g., Cruden 2000, Wyatt *et al.* 2000).

## Materials and Methods

Materials used in this study were collected in the field (Table 1). They were fixed with FAA (formaldehyde solution: acetic acid : 50% EtOH = 5 : 5 : 90) and stored therein until observed. Voucher specimens are kept in TI (University of Tokyo Herbarium) and in the Botanical Gardens, Osaka City University.

Flower visitors were observed in the field on *Apinagia* sp. 1, *Rhyncolacis* sp. 2 and *Weddellina squamulosa*. For *Dalzellia zeylanica*, living plants on rocks were collected and brought back to Japan and cultivated in an aquarium in a greenhouse at the Botanical Gardens, Osaka University, Osaka, at a temperature between 15 and 30°C, where pollination was observed.

There are a number of publications concerning pollination systems in Podostemaceae using the terms "self," "cross pollination," "autogamy," "cleistogamy," "wind," "insect pollination," and so on. In this study we employ only the terms autogamy, allogamy and cleistogamy, because we did not conduct crossing tests or tests of self-compatibility.

Three flowers per species were used for counts of the number of stamens, pollen grains and ovules, except in *Apinagia longifolia*, *A.* sp.1, *Mourera fluviatilis* and *Rhyncolacis* sp. 2, where only one flower of each was examined. For species with small stamens, all pollen grains from the whole stamen were counted, otherwise pollen grains from half of the stamen were

TABLE 1. Localities and voucher specimens of investigated species of Podostemaceae.

species	locality*	voucher
<i>Apinagia longifolia</i> (Tul.) v. Royen	G; Essequibo River, Kurupukari Falls	Kato & Imaichi GU-123
<i>A. sp.1</i>	G; Essequibo River, Gold fast Rapids	Kato & al. GU-21
<i>A. sp.2</i>	G; Essequibo River, Gold fast Rapids	Kato & al. GU-22
<i>Cladopus taiensis</i> Cusset	T; Nakhon Nayok, Nang Rong Waterfalls	Kato & Imaichi TL-103
<i>Dalzellia zeylanica</i> Wight	S; Peradeniya	Kato & al. SL-23
<i>Hanseniella heterophylla</i> Cusset	T; Kaeng Sopha waterfall, Tung Saleng Luang Nat. Park	Kato & al. TL-311
<i>Hydrobryum griffithii</i> Tul.	T; Doi Inthanon	Kato & Imaichi TL-65
<i>Malaccotristicha sp.</i>	T; Ubon Ratchathani, Yoddome Wildlife Sanctuary	Kato & al. TL-321
<i>Mniopsis sp.</i>	B; Rio de Janeiro, Rio Soberbo	Kato & al. BR-8
<i>Mourera fluviatilis</i> Aubl.	G; Essequibo River, Head Falls	Kato & al. GU-118
<i>Polypleurum elongatum</i> (Gardn.) Hall	S; Mahaweli Canga, Nawalapitiya	Kato & al. SL-17
<i>P. stylosum</i> (Wight) Hall	S; Mahaweli Ganga, Peradeniya	Kato & al. SL-20
<i>P. wallichii</i> (Brown ex Griffith) Cusset ex Kato	T; Pha Kluaïmai & Haew Suwat Waterfalls, Khao Yai Nat. Park, Nakhon Nayok	Kato & al. TL-55
<i>Rhyncolacis sp.1</i>	G; Ireng River, Kaitur Water Fall	Kato & al. GU-2
<i>R. sp.2</i>	G; Essequibo River, Head Falls	Kato & al. GU-5
<i>Synstylis micranthera</i> (v. Royen) Cusset	T; Kao Soi Dao, Chanthaburi	Kato & Imaichi TL-62
<i>Torrenticola queenslandica</i> (Domin) Domin ex Steenis	A; Queensland, Little Millstream Waterfalls	Kato & al. AU-101
<i>Tristicha trifaria</i> (Bory ex Willd.) Spreng.	B; Mato Grosso; Rio Serragem A; Northern Territory, Katherine Gorge	Kato & al. BR-9 Kato & al. AU-301
<i>Weddellina squamulosa</i> Tul.	G; Essequibo River, Head Falls	Kato & al. GU-3
<i>Zeylanidium subulatum</i> (Gardn.) Cusset	S; Peradeniya, Ivory Island	Kato & al. SL-18

\* Abbreviations are A: Australia, B: Brazil, G: Guyana, S: Sri Lanka, T: Thailand.

used. The pollen was spread onto a glass slide and stained with cotton blue in lacto-phenol. All pollen grains on the slide were counted. Dyads were counted as two pollen grains. The pollen/ovule ratio (P/O) was estimated by (number of pollen grains per flower) / (number of ovules) per flower. An average P/O value was obtained for each species (Table 2).

## Results

### *Floral behavior of Dalzellia zeylanica*

Fresh saxicolous plants with flower buds were brought into cultivation and kept submerged in an aquarium in December 1998. On January 19th 1999, the plants were raised to just above the water surface. At that time, the plants were still wet. Twenty days later, the peduncles began

to elongate. On February 19th, the plants were placed in the air and allowed to dry. On February 22nd the flowers opened simultaneously at 9.30 am (Fig. 1A). The stigmatic areas were feather-like papillae as in Graminae during pistil maturation. On the next morning (10.00 am, February 23rd), the papillae were still fresh. The stamens dehisced and were in close proximity to the stigmatic area, or sometimes even attached to the papillae. At that stage, we observed that pollen grains were shed and were trapped on the stigmatic areas when the flowers were gently touched. This observation suggested the possibility of allogamy through the action of wind in this species. At 10.00 am, February 24th, the papillae began to droop and the stamens recurved. We did not observe any visitors to the flowers during anthesis. The plants subse-

quently became dry. Two days later, the stamens were dry, and the peduncles became hard. About a month later (end of March) the capsules dehisced. We harvested the mature fruits, which were filled with fertile seeds. The seeds germinated readily a week after being sown, indicating that in *Dalzellia zeylanica* reproduction occurred autogamously and allogamously through the action of wind in cultivation. Willis (1902) reported that in nature the flowers were fertilized underwater and reproduction was cleistogamous (although not strictly so).

*Flowers and capsules of Polypleurum elongatum*  
We collected plants of *Polypleurum elongatum* that were submerged in the rapids of a stream. The flowers, which had already been pollinated, were distributed along the elongated roots distal to other roots with capsules. The roots were still fresh along their entire length (Fig. 2). If the flowers had developed to maturity under dry conditions like many other Podostemaceae, the plant bodies (roots) would have been drooping and decomposing, and the capsules should have formed basipetally, i.e., from the distal to the proximal part of the root. The acropetalous flower formation and fruit maturation therefore suggest that in *P. elongatum* the flowers bloom and are pollinated while submerged, as in *Cladopus nymanii*, *C. japonicus*, and *Podostemum ceratophyllum* (Möller 1899, Imamura 1928, Philbrick 1984). Pollination underwater is inconceivable in rapidly flowing water unless the stigmatic area and anthers are in close proximity to one another, which then would indicate cleistogamous autogamy.

*Flower visitors on Apinagia sp., Rhyncolacis sp. and Weddellina squamulosa*

The stamens of *Apinagia* and *Rhyncolacis* are corolla-like and resembles those of *Thalictrum* (Ranunculaceae). They spread horizontally and

are pink at anthesis (Fig. 1B). The stigmas emerge from the whorl of stamens after maturation of the anthers, but the stamens never touch the stigmatic areas. This floral developmental pattern does not suggest that the flowers are autogamous. *Apinagia* sp. 1 and *Rhyncolacis* sp. 2 reproduced allogamously through the action of insects in the field, where many flowers bloomed simultaneously as in other species. We observed many stingless bees, mainly *Melipona* sp. (Halictidae), *Trigona* spp. (Meliponini, Meliponinae, Apidae) (Fig. 1C), and other insects visiting the flowers of both species. The insects harvested pollen, which they stored in pollen baskets on their legs and frequently came into contact with the anthers and stigmatic areas.

The flowers of *Weddellina squamulosa* have relatively showy white petals, which appear to be attractive to insects. Although Grubert (1974) and Rutishauser (1997) described *W. squamulosa* as insect-pollinated, we did not observe any visitors to the flowers in the field. Flies sometimes landed on the plant body, probably attracted by the decomposing vegetative portion, but not on the flowers. Just before anthesis, the stigmatic area was in contact with the stamens, which had already dehisced (Fig. 3A). At the same time, some pollen grains had already germinated in the dehiscent anthers, indicating that the species may be autogamous.

#### *Pollen - ovule ratio*

A wide range of variation in the numbers of stamens, pollen grains per stamen, and ovules were observed in Podostemaceae (Table 2). For example, the number of pollen grains per flower in *Mourera fluviatilis* (ca. 0.8 million grains) was about 800 times more than in *Tristicha trifaria* (ca. 1000 grains), and the number of ovules per flower in *M. fluviatilis* (ca. 2800) was about 280 times more than in *Hanseniella hetero-*

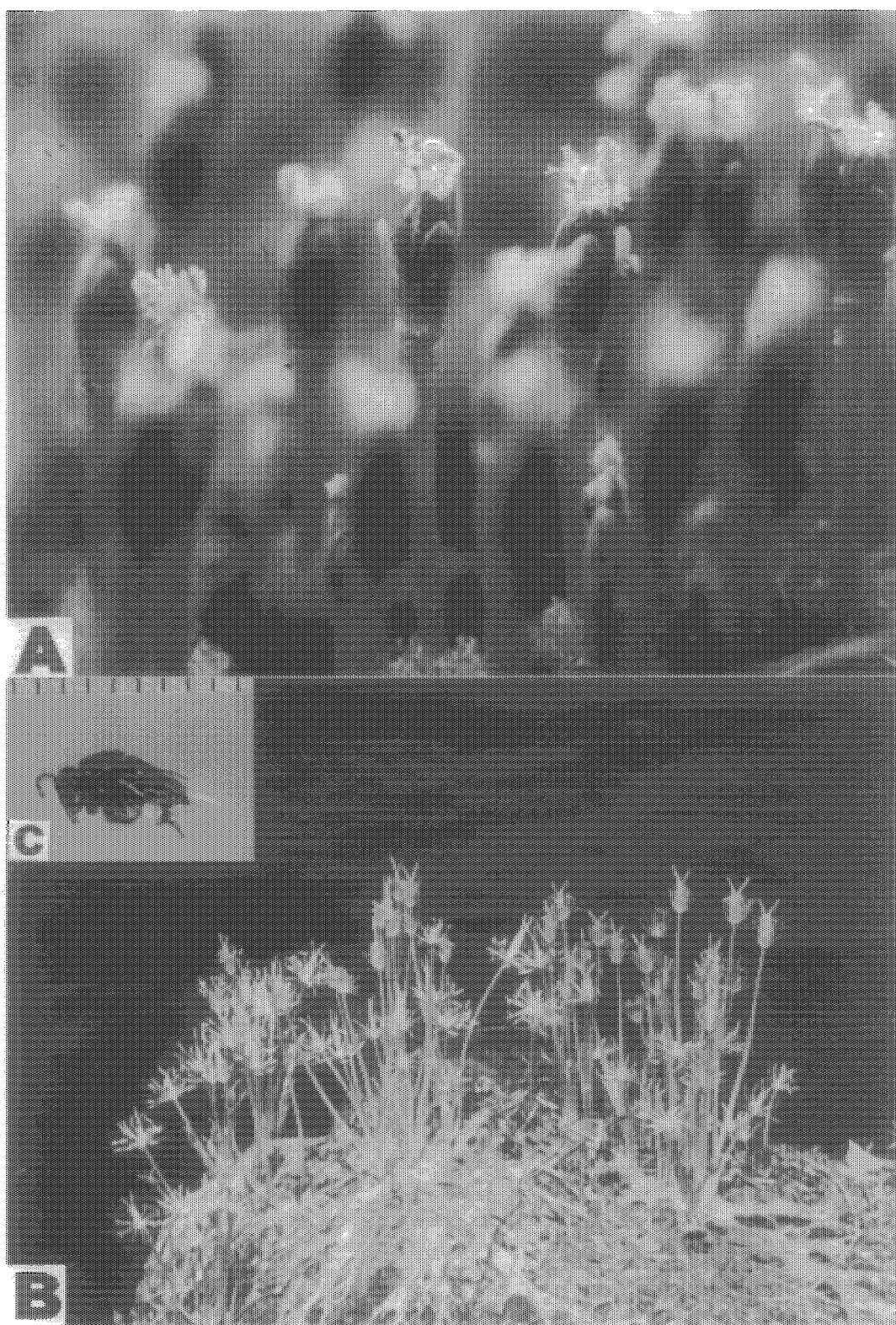


FIG. 1. Flowers of some Podostemaceae. A: *Dalzellia zeylanica*. ca. x 3. Note that the papillate stigmatic area is attached to stamens. B: *Rhyncolacis* sp. 2. ca. x 0.6. Note the stigmatic area far from stamens. C: Stingless bee, *Trigona* sp., Meliponini, Meliponinae, Apidae, captured on a flower of *Rhyncolacis* sp. 2. Scale, 1mm.

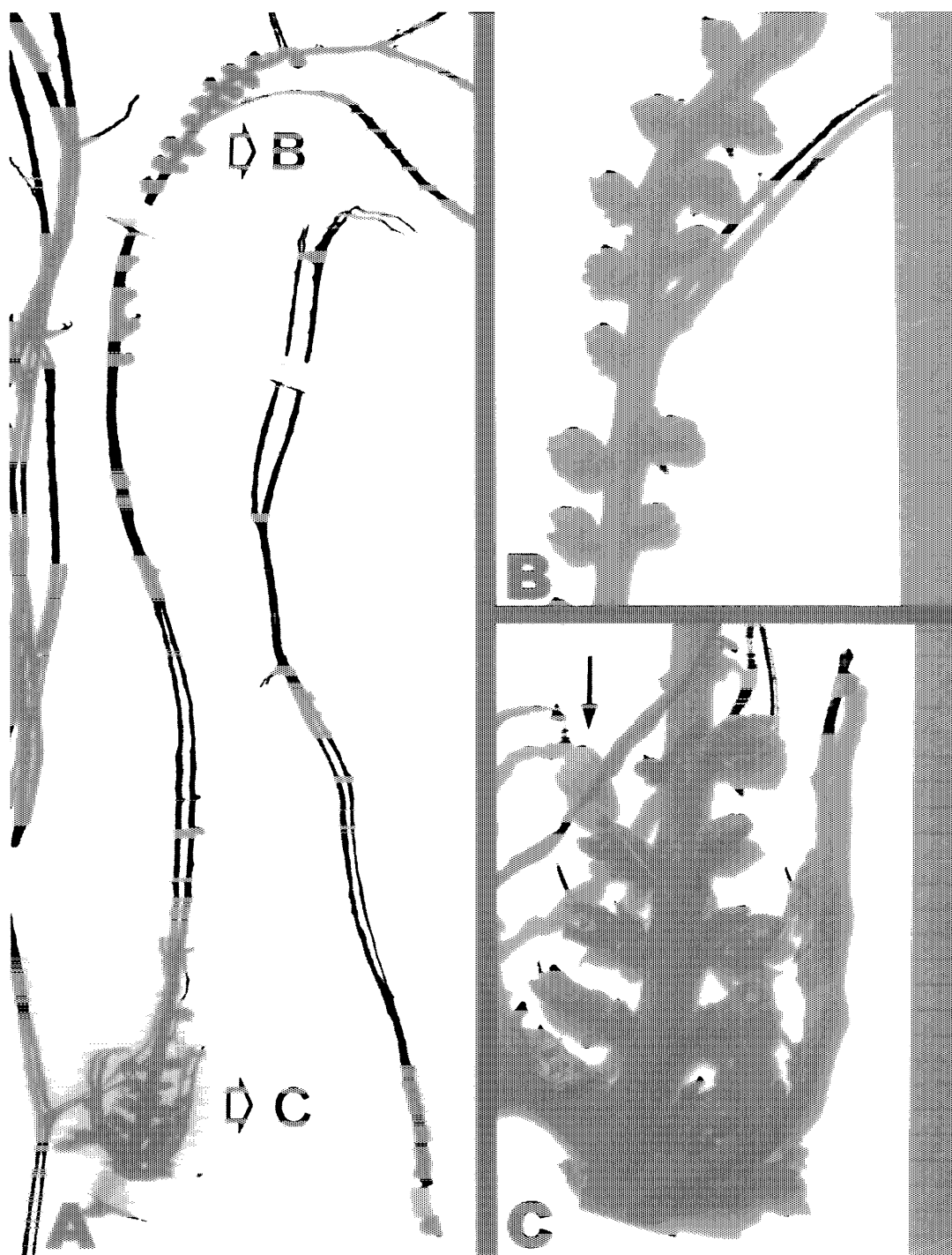


FIG. 2. Capsule and flower buds on an elongate root of *Polypleurum elongatum*. A: whole plant. Zoom up views of parts of plant body (arrow B and C) are shown in B and C, respectively. B: flower buds on the distal part of root. C: a capsule (arrow) and flower buds at the base of root. This specimen was collected from a rock at the bottom of stream. Unit scales in B and C, 1mm.

TABLE 2. P - O ratios of species of Podostemaceae examined.

Species*	stamen no.	pollen no. per stamen	ovule no.	log(P/O) (min.-max.)	pollination**
Tristichioideae					
<i>Dalzellia zeylanica</i>	3	4904-8010	202-235	1.924 (1.862-2.009)	AUTO + ALLO by wind
<i>Malaccotristicha</i> sp.	2	608-737	55-66	1.346 (1.344-1.348)	
<i>Tristicha trifaria</i> (BR-9)	1	965-1163	90-93	1.064 (1.029-1.100)	AUTO
<i>T. trifaria</i> (AU-301)	2-3	2328-3647	176-199	1.597 (1.423-1.731)	
Weddellinoideae					
<i>Weddellina squamulosa</i>	5-7	4618-6520	272-407	1.980 (1.837-2.050)	AUTO (and ALLO by insects (cf. Grubert 1974, Rutishauser 1997))
Podostemoideae					
<i>Apinagia longifolia</i>	18	27676	1515	2.517	
<i>A.</i> sp.1.	20	26864	1304	2.615	ALLO by bees
<i>A.</i> sp.2.	3-4	11676-13016	264-314	2.178 (2.095-2.248)	
<i>Cladopus taiensis</i> *	1	2294-2598	32-45	1.849 (1.761-1.906)	
<i>Hanseniella heterophylla</i> *	2	9100-10164	11-13	3.223 (3.194-3.255)	ALLO
<i>Hydrobryum griffithii</i> *	2	2802-3742	36-45	2.247 (2.095-2.308)	
<i>Mniopsis</i> sp.*	2	1428-2350	96-111	1.558 (1.474-1.626)	
<i>Mourera fluviatilis</i>	29	27636	2876	2.445	ALLO by bees (cf. Rutishauser 1997)
<i>Polypleurum elongatum</i> *	2	5206-7632	72-98	2.163 (2.045-2.230)	AUTO
<i>P. stylosum</i> *	2	5680-7792	51-81	2.312 (2.284-2.348)	
<i>P. wallichii</i> *	2	4598-4946	119-142	1.870 (1.827-1.892)	
<i>Rhyncolacis</i> sp.1.	7-8	11788-15862	212-299	2.628 (2.441-2.777)	
<i>R.</i> sp.2.	14	11783	534	2.490	ALLO by bees
<i>Synstylis micranthera</i> *	1	9768-13396	15-28	2.752 (2.680-2.814)	
<i>Torrenticola queenslandica</i>	1	13032-14992	66-89	2.256 (2.168-2.356)	
<i>Zeylanidium subulatum</i> *	2	11824-18268	77-97	2.538 (2.415-2.601)	

\* Species with dyads

\*\* Abbreviations are AUTO: autogamy, ALLO: allogamy

*phylla* (ca. 10). As a result, P/O ratios ranged from 11.6 ( $\log P/O = 1.066$ ) in *T. trifaria* to more than 1600 ( $\log P/O = 3.223$ ) in *H. heterophylla*. Comparison of P/O ratios with pollination systems and floral phenology we observed indicates that species with higher P/O ratios ( $\log P/O = 2.615$  in *Apinagia* sp. 1.,  $\log P/O = 2.490$  in *Rhyncholacis* sp. 2.) are cross pollinated by flower visiting insects, while those with lower P/O ratios ( $\log P/O = 1.928$  in *Dalzellia zeylanica*,  $\log P/O = 2.162$  in *Polypleurum elongatum*,  $\log P/O = 1.976$  in *Weddellina squamulosa*) are self pollinating.

### Discussion

There is great variation in the floral morphology of Podostemaceae, although the flowers are generally small and inconspicuous. The subfamily Tristichioideae (*Dalzellia*, *Indotristicha*, *Malaccotristicha*, *Terniopsis*, *Tristicha*) has radially symmetrical flowers with three, relatively large tepals, one to three stamens, and a trilobular ovary. Subfamily Weddellinoideae (*Weddellina*) has similar radially symmetrical flowers with five relative large tepals, five stamens, and a

bilobular ovary. In comparison, the subfamily Podostemoideae has zygomorphic or dorsiventral flowers, and the flowers of some Asian-Australian genera (*Cladopus*, *Hanseniella*, *Hydrobryum*, *Polypleurum*, *Synstylis*, *Torrenticola*) have two rudimentary, inconspicuous sepals, a simple or branched stamen, and a bilobular ovary. Some American genera (e.g., *Apinagia*, *Mourera*, *Rhyncholacis*) have showy flowers with colorful (pink, pink-purple) peduncles and a number of stamens with likewise colorful filaments. Sculthorpe (1967) considered the flowers of the least specialized genera, e.g., *Indotristicha*, to be insect pollinated and the specialized zygomorphic (dorsiventral) flowers to be associated with the loss of entomophily and adaptation to anemophily. Rutishauser (1997) considered wind pollination, besides autogamy and cleistogamy, to be typical in Tristichioideae, but the flowers of *Weddellina* (then assigned to the subfamily) are showy and insect-pollinated. In such American genera as *Apinagia*, *Mourera*, and *Rhyncholacis*, polyandry with showy stamens and peduncles is related to insect pollination; in Asian genera the small and inconspicuous flowers are wind

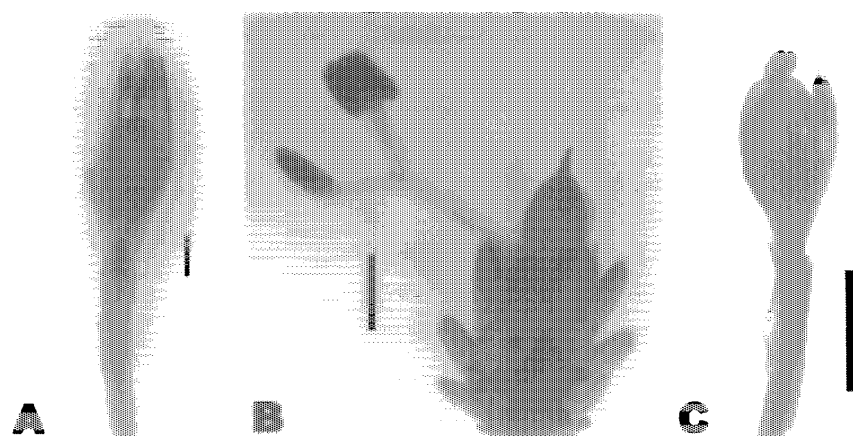


FIG. 3. Flowers of some Podostemaceae. A: *Weddellina squamulosa* just before anthesis. Some petals were removed. Note that the stigmatic area and stamens are in close proximity. Stamens are already dehiscent. B: *Hanseniella heterophylla* at anthesis. Note that the stigmatic area far from stamens, which are not yet dehiscent. C: *Tristicha trifaria* just before anthesis. Tepals were removed. Note that the stigmatic area and stamens are in close proximity. Bars, 1mm.



pollinated or self pollinated, or even cleistogamous before anthesis (Willis 1902, Rutishauser 1997). Floral zygomorphy in Podostemaceae is derived from radial symmetry, as seen in Tristichioideae (Willis 1902, Rutishauser 1997). Furthermore, it seems likely that cleistogamy was evolved to insure fertilization in flowers that are never elevated above the water in aquatic angiosperms (Sculthorpe 1967, Philbrick & Les 1996), as in some Podostemaceae (Möller 1899, Willis 1902, Philbrick 1984). The pollination systems in Podostemaceae, therefore, appear to be varied. This impression may have arisen from the scant evidence so far reported on reproduction in the family.

This paper is the first record of P/O ratios for a considerable number of species of Podostemaceae. There are some extreme examples in log P/O ratio values, such as in *Hanseniella heterophylla* (3.223) and *Tristichia trifaria* (BR-9) (1.066). *H. heterophylla* has very small flowers with a small number of large ovules. Such small flowers appear to be unattractive to flower visitors. The floral morphology where the stamens and stigmatic area are far from one another (Fig. 3B) suggests that autogamy is unlikely to occur. Instead, those species are probably allogamous. *Tristichia trifaria*, on the other hand, is considered to be autogamous based on the fact that its stigmatic areas and anthers are attached to each other (Fig. 3C). *Dalzellia zeylanica* produces stigmatic areas with feather-like papillae (Fig. 1A), which appear to be an adaptation to wind pollination, as in *Indotristicha ramosissima* (Rutishauser & Huber 1991). Pollen grains were observed to be trapped on the stigmatic areas. The anthers of *D. zeylanica* are in contact with stigmatic areas and pollination takes place autogamously. Rather high values of log P/O (1.924) may be a reflection of both pollination systems. It may be that the species with values of log P/O

higher than 2.5 are allogamous, while those with values lower than 2.0 are autogamous (Table 2). These inferences do not contradict Cruden's (1977, 2000) rule. One exception was reported by Philbrick & Novelo (1998), who showed that wind pollinated, mostly autogamous and sometimes outcrossing *Marathrum rubrum*, which has somewhat showy flowers, has a P/O ratio of 616 (log P/O = 2.782, ranging from 205-1319). Further analysis is required for this species.

Kita & Kato (2001) analyzed molecular phylogenetic relationships in Podostemaceae, and agreed with Engler's (1930) classification that the family consists of three subfamilies, Tristichioideae, Weddellinoideae, and Podostemoideae. According to them, the Podostemaceae have two major clades. One clade is composed of subfamily Tristichioideae, which is the base of the family, and the other consists of the Weddellinoideae and Podostemoideae. Weddellinoideae is sister to the Podostemoideae. In this study, the species of subfamilies Tristichioideae and Weddellinoideae were seen to be autogamous and autogamous + allogamous (Table 2). Rutishauser & Huber (1991) reported wind pollination for *Indotristicha ramosissima* (Tristichioideae) from a morphological viewpoint, while Willis (1902) proposed self pollination for this species from an ecological viewpoint. *Indotristicha ramosissima* forms a clade with *Dalzellia zeylanica* (Kita & Kato 2001), which is both autogamous and allogamous as mentioned above. It is therefore considered that *I. ramosissima* is probably fertilized through both mechanisms, as mentioned by Rutishauser (1997). There is a contradiction between our observations and previous reports (Grubert 1974, Rutishauser 1997) on the pollination system of *Weddellina squamulosa*. Even though insects carry pollen of this species, cleistogamous autogamy occurred before anthesis (Fig. 3A).

Probably, both means of pollination occur, as seen in *D. zeylanica*. In contrast, the species in subfamily Podostemoideae utilize various pollination systems; some are allogamous while others are autogamous (Table 2). It is an interesting phenomenon that species belonging to subfamilies Tristichoideae (*D. zeylanica*, *I. ramosissima* and *Tristicha trifaria*) and Weddellinoideae (*W. squamulosa*) show autogamy and autogamy + allogamy, while the species in subfamily Podostemoideae (*Apinagia* sp. 2, *Hanseniella heterophylla*, *Mourera fluviatilis*, *Polypleurum elongatum* and *Rhyncolacis* sp. 2) are autogamous or allogamous. Subfamily Tristichoideae is the primitive group in Podostemaceae, as mentioned above (Kita & Kato 2001). The possibility that the ancestor of Podostemaceae that first invaded the specialized aquatic habitat was autogamous should be tested by further analysis of pollination and breeding systems in nature. As reported by Philbrick (1984), various types of autogamy are associated with such stressful or marginal situations as the aquatic habitat.

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